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ABSTRACT OF THE DISCLOSURE

A chemiluminescent substrate of hydrolytic enzyme having the following general Formula I, as follows:

Lumi-M-P

5 Formula I

"Lumi" is а chemiluminescent moiety capable producing light (a) by itself, (b) with MP attached and (c) with M attached. Lumi includes, but is not limited to, chemiluminescent acridinium compounds (e.g. acridinium acridinium carboxyamides, acridinium thioesters and acridinium oxime esters) benzacridinium compounds, quinolinium compounds, isoquinolinium compounds, phenanthridinium compounds, and lucigenin compounds, acridans) or non-N-alkylated forms reduced (e.g., (e.g., acridines) of the above, spiroacridan compounds, and isoluminol compounds and the like. luminol compounds M is a multivalent heteroatom having at least one lone from oxygen, nitrogen and of electrons selected sulfur, directly attached to the light emitting moiety of Lumi at one end and to P at the other end. (When M alone is attached to Lumi to form Lumi-M, it does, of course, have either a proton or a counterion associated with it or is in the form of an ion.). P is a group that can be readily removed by hydrolytic enzymes, as discussed The light emitting moiety more detail hereinafter. of example, when Lumi well known. For acridinium compound or luminol, the light emitting moiety

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is the acridinium nucleus or phthaloyl moiety, respectively.

An enzymatic reaction having the following general reaction A, as follows:

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m HE}$ is a hydrolytic enzyme, such as phosphatase, glycosidase, peptidase, protease, esterase, sulfatase and quanidinobenzoatase. Lumi-M-P is а chemiluminescent hydrolytic of enzyme. Lumi-M is substrate chemiluminescent product having physical and/or chemical properties different from those of Lumi-M-P. Said physical and/or chemical properties include emission wavelength, quantum yield, light emission kinetics, fundamental net charge distribution, dipole moment, π -bond orders, free hydrophilicity, hydrophobicity/ apparent or solubility, affinity and other properties.

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